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Opposing Roles of Interferon-γ on CD4+ T Cell–Mediated Graft-Versus-Host Disease: Effects of Conditioning

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ABSTRACT

Although alloreactive T cells are required for the induction of graft-versus-host disease (GVHD), other factors can influence outcome in murine models of the disease. Lethal total body irradiation (TBI) conditioning regimens followed by reconstitution with allogeneic lymphohematopoietic cells results in the generation of donor anti-host cytotoxic T lymphocyte (CTL)-mediated solid organ (gut, liver, skin) destruction. In contrast, donor anti-host CTL-mediated hematopoietic failure is the primary cause of morbidity following sublethal TBI. To determine the role of interferon (IFN)-γ in graft-versus-host reactions against hematopoietic and solid organ targets, we used IFN-γ knockout mice as donors in both lethal TBI and bone marrow transplantation (BMT) rescue and sublethal TBI models. In this report, we show that CD4+ T cells from IFN-γ knockout (KO) mice resulted in accelerated GVHD after lethal TBI/BMT using a single major histocompatibility class II mismatch model. In marked contrast, the use of these same IFN-γ KO CD4+ donor cells in combination with sublethal TBI significantly ameliorated GVHD-associated mortality. In these recipients, severe anemia, bone marrow aplasia, and intestinal lesions were observed in the presence but not the absence of donor-derived IFN-γ. Administration of anti–IFN-γ antibodies to sublethally irradiated recipients of wild-type donor cells confirmed the role of IFN-γ depletion in CD4+ T cell–mediated GVHD. In conclusion, the extent of conditioning markedly affects the role of IFN-γ in GVHD lesions mediated by CD4+ T cells. In models using sublethal TBI, the absence of IFN-γ is protective from GVHD, whereas in lethal TBI situations, the loss is deleterious.

KEY WORDS

Interferon-γ • CD4+ T lymphocytes • Graft-versus-host disease • Radiation • Histocompatibility antigens class II

INTRODUCTION

The role of the conditioning regimen in allogeneic hematopoietic stem cell transplantation is 2-fold: to deplete the host hematopoietic and immune systems, thereby allowing the successful establishment of donor hematopoietic cells, and to facilitate eradication of cancer cells in patients with malignant disorders. A side effect of conditioning therapy is the induction of cellular damage and macrophage activation in many tissues within the body. In this environment, donor alloreactive T cells can be more easily sensitized to the host tissues. Both induction and severity of graft-versus-host disease (GVHD), through concomitant tissue damage, may be affected by the conditioning regimen [1].

Interferon (IFN)-γ is an inflammatory cytokine produced by T cells [2] and natural killer (NK) cells [3,4] in response to mitogenic or antigenic stimulation. The IFN-γ receptor is found on a variety of tissues. IFN-γ, through its cognitive receptor, exerts multiple effects on both the effector and target cells involved in GVHD. IFN-γ can increase the

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T-cell sensitization phase of GVHD through upregulation of major histocompatibility complex (MHC) classes I and II [5,6] on allogeneic host antigen-presenting cells. At the same time, IFN-γ can suppress T-cell proliferation [7] and functional responses [8] leading to immunosuppression and can cause lymphoid hypoplasia associated with GVHD [7]. IFN-γ can also act either directly or indirectly to regulate GVHD via induction of Fas [9] or nitric oxide [10,11], which can augment cellular cytotoxicity.

In recent studies using IFN-γ knockout (KO) mice donor lymphocytes infused into full MHC mismatched recipients conditioned with lethal total body irradiation (TBI), we have shown that the absence of donor IFN-γ accelerated GVHD [12], indicating that IFN-γ can be protective in lethal TBI models of GVHD. Here we extend these findings and examine the role of IFN-γ in $CD4^+$ T cell–mediated murine GVHD using different amounts of conditioning. We observed amelioration of the GVH reaction with IFN-γ–deficient $CD4^+$ donor cells when single MHC class II–mismatched recipient mice were conditioned with sublethal TBI—although these same donor cells had the ability to accelerate GVHD in a lethal TBI/bone marrow transplantation (BMT) model. The data indicate that IFN-γ has a differential effect on $CD4^+$ T cell–mediated GVH reaction that is dependent on the extent of the conditioning regimen used to prepare the recipient.

MATERIALS AND METHODS

Mice

C57Bl/6J (B6, H-2^b) mice with a mutation in a single MHC class II molecule designated B6.C.$H2^{bm12}$ (bm12), B6 IFN-γ KO mice, and B10.BR (H-2^k) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). B6 mice were purchased from either the Animal Production Area (National Cancer Institute–Frederick Cancer Research and Development Center [NCI-FCRDC]) or Jackson Laboratory. For some experiments, breeding pairs of C57BL/6 IFN-γ KO mice originally purchased from Jackson Laboratory were maintained at NCI-FCRDC. Recipient mice were females, 8 to 16 weeks of age at time of transplantation. Recipients within an individual experiment were age-matched. Animals were cared for humanely according to the US Public Health Policy on the Care and Use of Animals and the Guide for the Care and Use of Laboratory Animals. NCI-FCRDC and the University of Minnesota facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Induction of GVHD

For sublethal irradiation and purified lymph node (LN) T-cell transfer involving the B6 into bm12, recipient mice were irradiated with 600 cGy TBI by a ^{137}Cs source. Experiments were performed at both institutions (NCI-FCRDC and University of Minnesota Cancer Center) with irradiators delivering γ-irradiation at a dose rate of 212 cGy/min and 85 cGy/min, respectively. Purified B6 $CD4^+$ (10^6) LN T cells were injected into bm12 recipients. Purified T-cell preparations were obtained from single-cell suspensions of axillary, mesenteric, and inguinal LNs treated with antibodies to NK cells and $CD8^+$ cells and passaged through a goat anti-mouse and goat anti-rat immunoglobulin (Ig)–coated column or with a commercial enrichment antibody cocktail and column (R&D Systems, Minneapolis, MN). The inoculum was >90% $CD4^+$ cells as determined by flow cytometry. To determine the extent of chimerism of donor $CD4^+$ T cells in sublethally irradiated mice, 4 million purified $CD4^+$ LN cells were infused into B10.BR mice conditioned with 600 cGy (212 cGy/min). For lethal irradiation/BMT involving the B6-into-bm12 strain combination, bm12 recipients were irradiated with 800 cGy TBI by x-ray (41 cGy/min). Recipients were given purified B6 $CD4^+$ (3×10^5) LN T cells along with 2×10^7 bone marrow T cells treated with anti-Thy 1.2 (antibody 30-H-12, rat IgG2b, provided by Dr. David Sachs, Charlestown, MA) and complement (Nieffenegger, Woodland, CA).

In some experiments, bm12 mice also received neutralizing antibodies to IFN-γ [13] (clone R4-6A2) or an isotype control (rat IgG) (PharMingen, San Diego, CA) at 300 μg/0.5 mL phosphate-buffered saline injected twice a week, intraperitoneally, from day 1 to 21 after $CD4^+$ T-cell transfer. This dosage of antibody has been previously shown to inhibit IFN-γ responses in vivo [12,14,15].

All cell infusions were by caudal vein injection in 0.5 mL volume. There were 8 to 10 animals per treatment group in each experiment. Survival studies were performed 3 to 4 times. Mice were monitored and weighed weekly. All moribund animals were euthanized.

Hematocrits

As an additional assessment of donor anti-host cytotoxic T lymphocyte (CTL)-mediated bone marrow destruction, blood was collected from mice by intraorbital puncture with heparinized microcapillary tubes for recipient blood hematocrit determination, an indicator of bone marrow aplasia. The tubes were centrifuged in a microhematocrit centrifuge for 5 minutes and the packed red cell volume was determined. Hematocrits were performed on 4 to 8 animals for each treatment group.

Flow Cytometry

Donor lymphocyte chimerism was assessed by the measurement of B6 (H-2^b) $CD4^+$ cells in the spleens of B10.BR (H-2^k) mice 7 days after purified $CD4^+$ LN cell infusion. Splenocytes were labeled with $CD4^-$ phycoerythrin and H-$2K^b$- or H-$2K^k$-fluorescein isothiocyanate murine antibodies (mAbs), obtained from PharMingen. Irrelevant mAb (mouse IgG_{2a}; PharMingen) control values were subtracted from values obtained with relevant mAbs. Ten thousand cells per determination were analyzed by 2-color flow cytometry using a FACScan (Becton Dickinson, San Jose, CA). Cells were gated for lymphocytes based on forward- and side-scatter settings.

Granulocyte/Macrophage–Colony-Forming Units

Effects on hematopoietic progenitors were assessed by the measurement of myeloid progenitors in the bone marrow and spleens of recipient mice. Mice were assessed at various time points. For each time point, 3 to 5 mice per group were analyzed. Spleen and bone marrow cells were washed and suspended in Iscove's modified Dulbecco's medium with 5% fetal bovine serum, 1% L-glutamine, and antibiotics. Cells

were plated in 1.1% methylcellulose (Fisher Scientific, Pittsburgh, PA) in triplicate 35-mm petri dishes (Falcon; Becton Dickinson, Lincoln Park, NJ) at a concentration of 0.5 to 1.0×10^6 spleen cells or 0.5 to 1.0×10^5 bone marrow cells per plate. Colony formation was stimulated with 10 ng/mL recombinant murine granulocyte/macrophage–colony-stimulating factor (rmGM-CSF) (Amgen, Thousand Oaks, CA) and 20 ng/mL recombinant murine interleukin (rmIL)-3 (Biological Response Modifiers Program Repository, Frederick, MD). Plates were incubated at 37°C for 7 days in 5% CO_2 with 100% humidity. Colonies (>50 cells) were enumerated on a Nikon stereo microscope (Nikon, Melville, NY).

Histology

To assess mice for evidence of GVHD-induced tissue destruction, tissues from recipient mice were placed in 10% formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Small intestines were prepared for histological evaluation using the Swiss roll technique. Tissue samples were collected 14 days after sublethal irradiation and 13 days after infusion of $CD4^+$-enriched LN cells. Liver, small intestine, and colon sections prepared from 3 animals per treatment group were assessed by a veterinary pathologist. Histopathologic changes were graded as minimal, mild, moderate, or severe and as focal, multifocal, or diffuse.

Statistical Analyses

Survival data were plotted by the Kaplan-Meier method and analyzed by the log-rank test. Two-way analysis of variance was used to compare time and treatment. Group comparisons were made with Student *t* test. A *P* value of <0.05 was considered significant.

RESULTS

IFN-γ–Deficient $CD4^+$ T Cells Accelerate GVHD Morbidity in Lethally Irradiated MHC Class II Only Disparate Recipients

We have previously reported that the absence of IFN-γ, which is produced by donor splenocytes, accelerates mortality in 2 distinct fully allogeneic mouse strain combinations involving lethal TBI and bone marrow cell rescue: the BALB/c ($H\text{-}2^d$) into B6 ($H\text{-}2^b$) model, which is disparate at the MHC and multiple minor histocompatibility antigens, and the B6 into B10.BR ($H\text{-}2^k$) model, which is disparate only at MHC [12]. To determine whether donor IFN-γ KO regulates $CD4^+$ T cell–mediated GVHD mortality, we transplanted purified $CD4^+$ LN T cells from either wild-type or IFN-γ KO B6 donors, along with T-cell–depleted bone marrow cells, into lethally irradiated (800 cGy; x-ray) bm12 (disparate at class II) recipients. When 300,000 $CD4^+$ cells were transplanted, there was a significant acceleration of GVHD-associated death in the absence of donor IFN-γ (Figure 1A). Profound weight loss was observed before mortality in recipients of IFN-γ KO $CD4^+$ cells (Figure 1B). These observations were confirmed in experiments using another conditioning source (900 cGy by ^{137}Cs; data not shown). This result demonstrates that IFN-γ is protective in $CD4^+$ T cell–mediated GVHD in models using lethal TBI and supports previous studies using whole populations of T cells [12].

Absence of Donor IFN-γ Results in Amelioration of $CD4^+$ T-Cell GVHD in a Sublethal TBI Model

B6 $CD4^+$ T cells mediate profound bone marrow aplasia and subsequent mortality when transferred into sublethally irradiated bm12 mice [16]. To determine the role of IFN-γ on T cell–mediated bone marrow failure, we used the sublethal TBI model for the $CD4^+$ T cell–mediated GVH reaction. Recipient (bm12) mice received a sublethal dose of TBI (600 cGy) followed by adoptive transfer of $CD4^+$ LN T cells from either wild-type or IFN-γ KO mice. Although we initially predicted that IFN-γ–deficient donor T cells would accelerate morbidity because of increases in allospecific GVH reactions as seen when lethal TBI was used, surprisingly, the absence of IFN-γ in $CD4^+$ T cells markedly ameliorated GVH-associated mortality (Figure 2). To demonstrate that the effects of using the IFN-γ KO cells were not due to developmental changes in the T-cell compartment of these mice, we performed the same studies using wild-type T cells and neutralizing antibodies to IFN-γ. Administration of neutralizing IFN-γ antibody to recipients of wild-type donor cells also resulted in protection from GVHD and confirmed the observations with IFN-γ–deficient T cells (Figure 2). These results indicated that in marked contrast to the data using lethal TBI/BMT, the loss of IFN-γ is protective in sublethal models of GVHD.

Failure of IFN-γ–deficient $CD4^+$ T cells to engraft in recipients following sublethal TBI would be consistent with improved outcome and 1 potential mechanism by which IFN-γ could potentiate GVHD. To assess this possibility, we determined the extent of donor T-cell chimerism in recipients after sublethal TBI. Because antibodies are not available to discriminate between B6 donor and bm12 recipient T cells, we infused B6 wild-type or IFN-γ KO $CD4^+$-enriched LN cells into MHC class I– and class II–disparate recipients (B10.BR) after sublethal TBI. IFN-γ KO $CD4^+$ donor cells were present at a higher number in the spleens at day 7 after cell transfer compared with wild-type $CD4^+$ donor cells in the recipient mice (Table 1). Taken together, these results suggest that IFN-γ plays a critical role in promoting $CD4^+$ T cell–mediated mortality in GVHD models using sublethal TBI without compromising donor T-cell chimerism.

Loss of IFN-γ Production by $CD4^+$ T Cells Confers Protection From Allospecific Induction of Anemia and Bone Marrow Aplasia in Sublethal TBI Models

Mortality is associated with profound anemia in the $CD4^+$/MHC class II–disparate sublethal TBI model. Sublethally irradiated recipient mice were anemic 14 days after $CD4^+$ wild-type (B6) T-cell infusions as determined by hematocrits (Table 2). However, recipients of donor IFN-γ KO $CD4^+$ T cells exhibited only a mild anemia associated with the TBI and did not significantly differ from control mice that received irradiation without subsequent $CD4^+$-enriched LN-cell transfer.

Hematologic status of bone marrow and spleen were assessed at various time periods early posttransfer in recipients of $CD4^+$-enriched LN T cells to confirm the absence of hematopoietic cell destruction by IFN-γ–deficient cells. Total nucleated cell content and committed hematopoietic progenitors as measured by the colony-forming

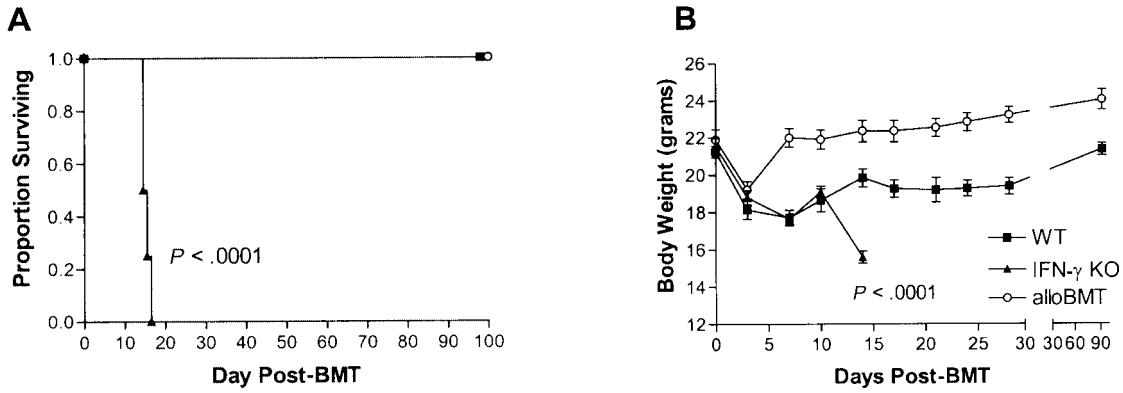

Figure 1. Interferon (IFN)-γ is protective for recipients of lethal total body irradiation (TBI)/bone marrow transplantation (BMT) in CD4⁺-mediated acute lethal graft-versus-host disease (GVHD). A, Recipient mice (bm12) received 800 cGy TBI followed by administration of donor T-cell–depleted bone marrow cells alone (alloBMT) or in combination with 3×10^5 B6 wild-type (WT) or B6 IFN-γ knockout (KO) CD4⁺-enriched lymph node cells (n = 8/group). GVHD morbidity was accelerated in recipients of IFN-γ–deficient donor cells ($P < .0001$). B, Weights of the animals that received lethal TBI followed by BMT as depicted in A. Recipients of IFN-γ KO CD4⁺ lymph node cells exhibited significant ($P < .001$) weight loss at day 14 after cell transfer relative to recipients of WT cells.

units–granulocyte/macrophage (CFU-GM) assay were examined. Recipients of wild-type CD4⁺-enriched LN T cells exhibited a severe loss of cells (Figure 3) in the bone marrow beginning on day 9 after transfer and in the spleen by day 13. This was also associated with a profound loss of myeloid progenitors (Figure 4). In contrast, recipients of IFN-γ KO CD4⁺-enriched LN T cells exhibited no inhibition of myeloid progenitor recovery and only a mild depression in bone marrow cellularity compared with similarly irradiated animals. The depression in splenic cellularity and myeloid progenitors observed in recipients of wild-type donor cells was not observed in recipients of IFN-γ KO donor cells. Overall, these findings suggest that alloreactive CD4⁺ T cells induce a loss of myeloid progenitors and subsequent progressive bone marrow and splenic aplasia, primarily through an IFN-γ–dependent mechanism.

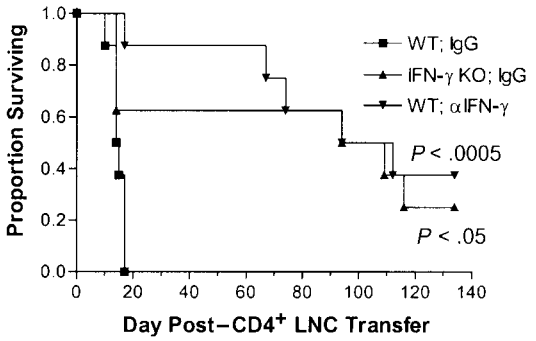

Figure 2. Interferon (IFN)-γ ameliorates graft-versus-host (GVH) mortality in recipients of sublethal total body irradiation (TBI) and CD4⁺ T cells. Recipient mice (bm12) received 600 cGy TBI followed by administration of B6 or B6 IFN-γ knockout (KO) donor T cells. Neutralizing IFN-γ antibody or irrelevant rat immunoglobulin (Ig) was administered as described in "Materials and Methods." One million (10^6) donor CD4⁺-enriched lymph node cells (LNCs) (>95% purity) were administered to recipients (n = 8). Survival of recipients of donor IFN-γ KO cells or B6 donor cells and IFN-γ antibody is significantly prolonged ($P < .05$ and $P < .0005$, respectively) compared with control. WT indicates wild type.

Loss of IFN-γ Production by CD4⁺ T Cells Confers Protection From Intestinal Lesions in Sublethal TBI, MHC Class II–Disparate Model of GHVD

Although morbidity is primarily associated with hematopoietic failure in the bm12 recipients of sublethal TBI and CD4⁺ T cells, it has been shown that the gut and liver are also targets for GVHD in this model [17]. In our studies, histological examination of the large and small intestines of the animals on day 13 after cell transfer revealed evidence of GVHD-associated pathology (Table 3 and Figure 5). In the small intestine, recipients of wild-type CD4⁺-enriched LN cells had mild multifocal crypt cell hyperplasia with the presence of apoptotic crypt epithelium. The lamina propria had infiltrates of mononuclear cells and occasional neutrophils. In comparison, only 1 of 3 recipients of IFN-γ KO CD4⁺-

Table 1. *Donor CD4⁺-Enriched T-Cell Chimerism in Spleens of MHC-Disparate Recipient Mice**

		Donor CD4⁺ T Cells	
Donor	Recipient	Frequency, %	Per Spleen, $\times 10^4$
B6 wild type	B10.BR	0.13 ± 0.7	1.6 ± 0.9
B6 interferon-γ knockout	B10.BR	2.89 ± 0.41†	39.7 ± 0.6†

*B10.BR recipient mice received 600 cGy total body irradiation followed by administration of 4×10^6 B6 or B6 interferon-γ knockout donor CD4⁺-enriched lymph node cells (>90% purity). No CD8⁺ cells were detected in the cell inocula. Spleens were analyzed for the presence of donor-derived T cells 7 days after cell transfer. Each treatment group contained 4 animals. MHC indicates major histocompatibility complex.

†Significantly different from B6 wild type, $P < .001$ (Student t test).

Table 2. *Hematologic Parameters Following Induction of GVH Reaction**

Experiment and Donor	Antibody Treatment	Recipient	HCT, % Day 14	HCT, % Day 28
Experiment 1				
B6 WT	**Rat IgG**	**bm12**	**13.2 ± 4.0**	**NS**
B6 IFN-γ KO	**Rat IgG**	**bm12**	**36.1 ± 4.5†**	**46.2 ± 4.4**
B6 WT	**Anti–IFN-γ**	**bm12**	**24.4 ± 4.2‡**	**40.3 ± 7.6**
Experiment 2				
B6 WT	**None**	**bm12**	**15.6 ± 1.5**	**NT**
B6 IFN-γ KO	**None**	**bm12**	**35.3 ± 1.3†**	**NT**
None	**None**	**bm12**	**39.0 ± 2.2†**	**NT**

*Recipient mice received 600 cGy total body irradiation (TBI) followed by administration of B6 or B6 interferon (IFN)-γ KO donor T cells. Neutralizing IFN-γ antibody or irrelevant rat immunoglobulin was administered as described in "Materials and Methods." Results from representative experiments are presented. One million (10^6) donor CD4⁺-enriched lymph node cells (LNC) (>95% purity) were administered to bm12 recipients. Control animals received TBI but did not receive LNCs. Each treatment group contained 4 to 8 animals. GVH indicates graft-versus-host; HCT, hematocrit; WT, wild type; Ig, immunoglobulin; NS, no survivors; KO, knockout; NT, not tested.

†Significantly different from B6, $P < .0005$ (Student t test).

‡Significantly different from B6, $P < .001$ (Student t test).

enriched LN cells had minimal crypt apoptosis of the small intestine. No inflammation or crypt hyperplasia was observed in these animals. The small intestines of recipients of sublethal TBI without T-cell infusion appeared normal at this time point. Similar observations were made in the colon on day 13 after LN-cell transfer. All recipients of sublethal TBI and wild-type CD4⁺-enriched LN cells displayed multifocal crypt cell hyperplasia and apoptosis in colonic crypts, whereas the recipients of IFN-γ KO CD4⁺-enriched LN cells and sublethal TBI controls appeared normal. No significant differences between the 3 treatment groups were observed in representative liver sections of these animals (data not shown). These results show that in this sublethal TBI model involving a single MHC class II mismatch, intestinal GVHD is also associated with the ability of donor cells to produce IFN-γ. Thus, even solid organ toxicity in CD4⁺-mediated GVHD is ameliorated in the absence of IFN-γ in sublethal TBI models.

DISCUSSION

The results presented here demonstrate that IFN-γ plays both critical and opposing roles in CD4⁺ T cell–mediated GVH reactions, depending on the extent of conditioning of the recipient. In mice conditioned with lethal TBI followed by MHC class II–disparate BMT, IFN-γ is protective. However, in mice conditioned with sublethal TBI followed by adoptive transfer of MHC class II–disparate CD4⁺ T cells, IFN-γ production by donor cells is deleterious.

The role of IFN-γ in alloresponsiveness is complex and at times contradictory. In addition to a direct inhibition of hematopoiesis by IFN-γ, myelosuppression by IFN-γ may be augmented through the induction of Fas [9] and MHC [5,6]. Elevated MHC surface expression could enhance targeting of hematopoietic stem cells and progenitors for allogeneic T-cell destruction by pathways that include Fas/FasL. Whereas MHC class I molecules are expressed on all nucle-

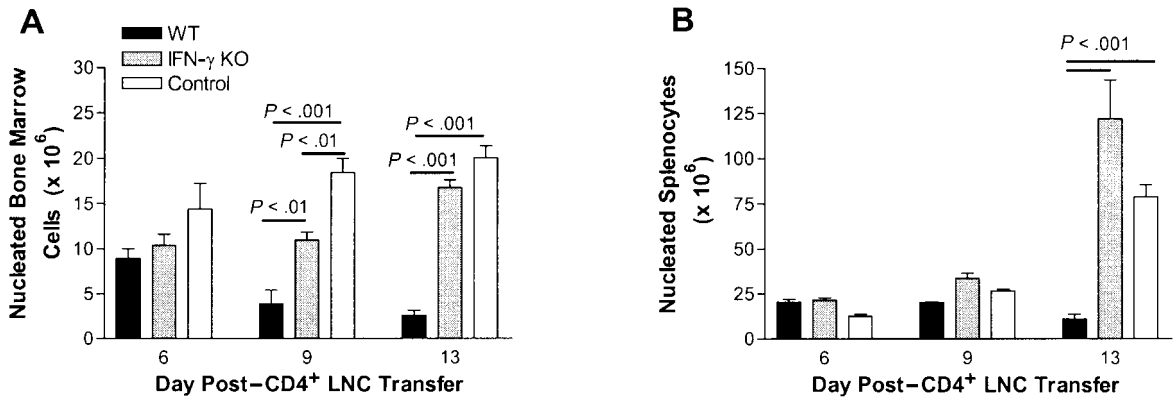

Figure 3. Severe bone marrow and splenic aplasia in recipients of wild-type (WT) but not interferon (IFN)-γ–deficient major histocompatibility complex (MHC)–disparate CD4⁺-enriched lymph node cells (LNCs). Recipient bm12 mice received 600 cGy total body irradiation (TBI) 1 day before infusion of B6 (■) or B6 IFN-γ knockout (KO) (■) donor T cells. Control animals (□) received TBI without subsequent cell transfer. The bone marrow and spleen cellularities were analyzed on days 6, 9, and 13 after CD4⁺ T-cell transfer. Progressive loss of bone marrow (A) and splenic (B) total nucleated cell content is observed following sublethal TBI and administration of WT but not IFN-γ–deficient MHC-disparate LNCs.

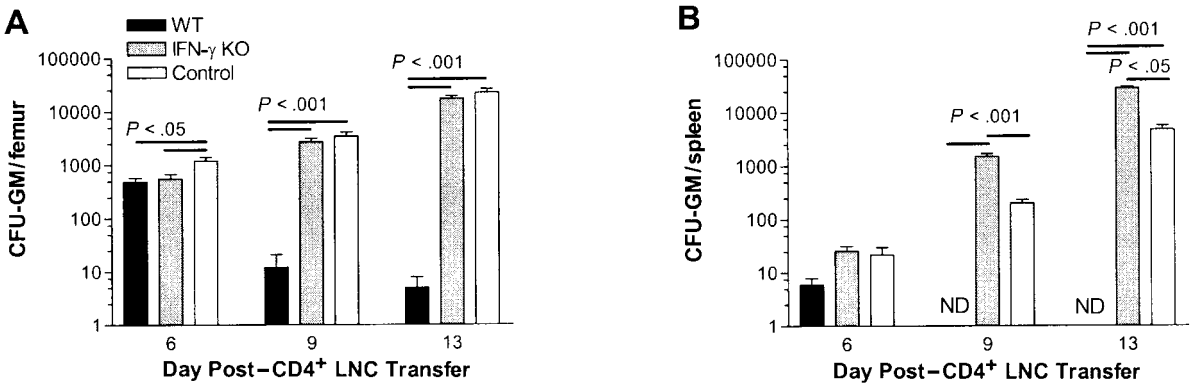

Figure 4. Wild-type (WT) but not interferon (IFN)-γ–deficient major histocompatibility complex (MHC)-disparate $CD4^+$-enriched lymph node cells (LNCs) suppress host hematopoietic progenitors. Recipient bm12 mice received 600 cGy total body irradiation (TBI) 1 day before infusion of B6 (■) or B6 IFN-γ knockout (KO) (▩) donor T cells. Control animals (□) received TBI without subsequent cell transfer. The hematopoietic progenitors in the bone marrow and spleen were analyzed on days 6, 9, and 13 after $CD4^+$ T-cell transfer. Progressive loss of bone marrow (A) and splenic (B) colony-forming units–granulocyte/macrophage (CFU-GM) is observed following sublethal TBI and administration of WT but not IFN-γ–deficient MHC-disparate LNCs. ND, not detected.

ated cells, MHC class II molecules are transiently expressed on developing hematopoietic precursors [18] and subsequently expressed on classical antigen-presenting cells. Donor $CD4^+$ T cells, capable of producing IFN-γ, suppress allogeneic hematopoiesis in an MHC class II–specific action, because it has been shown that engraftment of donor-derived bone marrow cells is not impaired [16]. In this report, we find that donor-derived IFN-γ also promotes MHC class II–specific allogeneic hematopoietic and gut tissue destruction after sublethal TBI. Interestingly, absence of IFN-γ does not significantly protect against allospecific $CD8^+$ T cell–mediated hematopoietic aplasia in a sublethal TBI model (data not shown), suggesting that $CD8^+$ T cells may use other pathways in GVHD.

MHC class II incompatibility alone is sufficient to induce intestinal GVHD [19-21], although lethality from GVHD, presumably from gut damage, requires lethal TBI in these MHC class II–disparate transplantations [21]. The combination of irradiation-induced tissue damage may induce tumor necrosis factor and other mediators that result in GVHD without the need for IFN-γ.

We and others have investigated the role of IFN-γ in GVHD. Brok and colleagues [22,23] have shown that the administration of IFN-γ in a full MHC-disparate lethal TBI/BMT model can ameliorate GVHD. Conversely, it has been shown that neutralization of systemic IFN-γ or the use of IFN-γ–deficient donor cells in lethal TBI/BMT models—with major or minor histocompatibility antigen differences—accelerates disease [12,24,25]. Together, these studies demonstrate that donor-derived IFN-γ has a protective function in acute GVHD, and the addition of exogenous IFN-γ potentiates this activity. In contrast, Ellison et al. [26] used a parent-into-F1 hybrid model to investigate the role of IFN-γ. They found that IFN-γ was important for mortality in their model, although recipients of IFN-γ–deficient T cells developed disease that was more characteristic of chronic GVHD. Their model differs from the previous studies in 2 aspects. The recipients in that model were not conditioned with TBI and the recipient T cells could not reject donor grafts; both aspects may have contributed to their findings. In agreement with our observations with IFN-γ–deficient $CD4^+$ T cells and sublethal irradiation, Ellison and colleagues not only

Table 3. *Histopathologic Evaluation of Intestinal Tissues on Day 13 Following Induction of GVH Reaction**

	WT $CD4^+$ LNC			IFN-γ KO $CD4^+$ LNC			Irradiation Control		
Animal	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Small intestine	**N**	**N**	**N**			**N**	**N**	**N**	**N**
Crypt cell hyperplasia	**2+ MF**	**2+ MF**	**2+ MF**						
Inflammation, subacute	**2+ MF**	**1+ MF**	**1+ MF**						
Apoptosis, crypt	**2+ MF**	**2+ MF**	**2+ MF**	**1+ MF**					
Globular leukocytes				**1+ MF**	**1+ MF**				
Colon				**N**	**N**	**N**	**N**	**N**	**N**
Crypt cell hyperplasia	**1+ MF**	**1+ MF**	**1+ MF**						
Inflammation, subacute			**1+ MF**						
Apoptosis, crypt	**1+ MF**	**1+ MF**	**2+ MF**						

*Grading information: 1+, minimal; 2+, mild; 3+, moderate; 4+, severe; N, no significant lesions; MF, multifocal. GVH indicates graft-versus-host; WT, wild type; LNC, lymph node cell; IFN, interferon; KO, knockout.

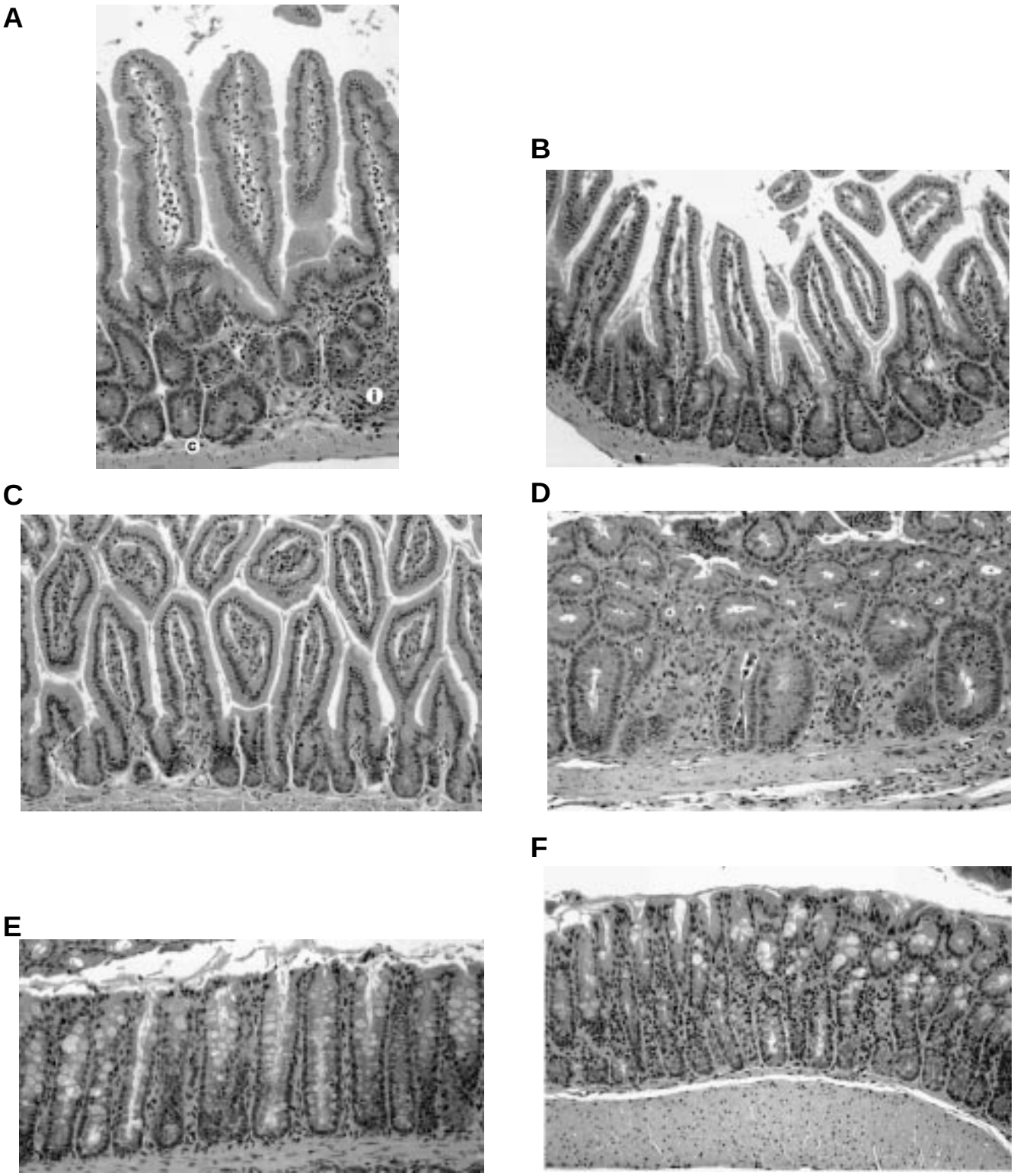

Figure 5. Representative photomicrographs showing protection from graft-versus-host (GVH)-associated intestinal damage with interferon (IFN)-γ–deficient $CD4^+$ donor T cells after sublethal total body irradiation (TBI). Recipient bm12 mice received 600 cGy TBI 1 day before infusion of B6 or B6 IFN-γ knockout (KO) donor T cells. Control animals received TBI without subsequent cell transfer. The small intestine and colon were collected and analyzed on day 13 after $CD4^+$ T-cell transfer. A, Small intestine from a recipient of B6 WT $CD4^+$ cells illustrating crypt cell hyperplasia (c), apoptotic crypt cells, and inflammation (i). B, Small intestine from a recipient of B6 IFN-γ KO $CD4^+$ cells with minimal crypt cell apoptosis. C, Small intestine from a recipient of sublethal TBI without subsequent cell transfer. No lesions. D, Colon from a recipient of B6 wild-type (WT) $CD4^+$ cells illustrating diffuse crypt cell hyperplasia. E, Colon from a recipient of B6 IFN-γ KO $CD4^+$ cells. No lesions. F, Colon from a recipient of sublethal TBI without subsequent cell transfer. No lesions.

observed diminished acute GVHD but greater expansion of donor-derived IFN-γ–deficient $CD4^+$ as well as $CD8^+$ T cells. Using a different model of acute GVHD, we have extended their observations to demonstrate that under nonmyeloablative preparative regimens, IFN-γ worsens acute GVHD. Further, we have found that the opposing roles of IFN-γ under different conditioning regimens are due to its activity in donor $CD4^+$ and not $CD8^+$ T cells.

Although we demonstrate that the opposing roles of IFN-γ in acute GVHD are dependent on the intensity of the conditioning regimen, this phenomenon is still not well understood. Induction of inflammatory cytokines by high-dose irradiation may be able to compensate for the immunostimulatory activities of IFN-γ. Loss of an autocrine immunosuppressive activity would then predominate as the mechanism for accelerated morbidity in the lethal TBI/BMT model. We have shown that adoptively transferred B6 IFN-γ KO $CD4^+$ T cells are present in greater numbers in the spleens of B10.BR mice prepared with sublethal TBI (Table 1) than are cells from wild-type donors. Although we cannot rule out that the absence of donor IFN-γ provides an advantage in evading rejection, 2 lines of evidence support increased survival of IFN-γ KO cells. First, in the article by Ellison et al. [26], using a parent-into-F1 model where recipient NK cells but not T cells can reject donor cells, there is greater expansion of donor-derived IFN-γ KO $CD4^+$ T cells compared with cells from wild-type donors. Second, IFN-γ KO splenocytes have been reported to exhibit sustained proliferation in response to mitogen and alloantigen [27].

The use of nonmyeloablative conditioning regimens for BMT has recently been under intense scrutiny. However, compared with transplants prepared with high-dose therapy, there is a greater concern for the establishment of donor chimerism. In the nonmyeloablative transplantation setting, the absence or blockade of IFN-γ in collaboration with MHC class II–mismatched grafts may diminish intestinal GVHD but at the expense of anti-host hematopoietic action. In a similar scenario, IFN-γ may enhance $CD4^+$-enriched delayed lymphocyte infusion activity against both normal and leukemic host hematopoietic progenitor cells [28,29].

In conclusion, acute lethal GVHD is accelerated in the absence of IFN-γ in an MHC class II–mismatched lethal TBI/BMT model. However, the absence of donor IFN-γ results in amelioration of GVH-associated mortality when sublethal levels of TBI are used as conditioning therapy.

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